

NATIONAL TOXICOLOGY PROGRAM  
Technical Report Series  
No. 417



TOXICOLOGY AND CARCINOGENESIS  
STUDIES OF *p*-NITROPHENOL  
(CAS NO. 100-02-7)  
IN SWISS-WEBSTER MICE  
(DERMAL STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
National Institutes of Health

## FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-1371).

NTP TECHNICAL REPORT  
ON THE  
TOXICOLOGY AND CARCINOGENESIS  
STUDIES OF *p*-NITROPHENOL  
(CAS NO. 100-02-7)  
IN SWISS-WEBSTER MICE  
(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM  
P.O. Box 12233  
Research Triangle Park, NC 27709

April 1993

NTP TR 417

NIH Publication No. 93-3148

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
National Institutes of Health

## CONTRIBUTORS

### **National Toxicology Program**

*Evaluated and interpreted results and reported findings*

C.J. Alden, Ph.D.  
G.A. Boorman, D.V.M., Ph.D.  
D.A. Bridge, B.S.  
S.L. Eustis, D.V.M., Ph.D.  
T.J. Goehl, Ph.D.  
R.A. Griesemer, D.V.M., Ph.D.  
J.K. Haseman, Ph.D.  
R.D. Irwin, Ph.D.  
G.N. Rao, D.V.M., Ph.D.  
C.C. Shackelford, D.V.M., M.S., Ph.D.  
D.B. Walters, Ph.D.  
K.L. Witt, M.S., Oak Ridge Associated Universities

### **Hazleton Laboratories**

*Conducted studies, evaluated pathology findings*

G.W. Wolfe, Ph.D., Principal Investigator  
R.H. Cardy, D.V.M.

### **Experimental Pathology Laboratories, Inc.**

*Provided pathology quality assessment*

J.F. Hardisty, D.V.M., Principal Investigator  
S. Neuenschwander, M.S., D.V.M.

### **Integrated Laboratory Systems**

*Performed quality assurance audits*

J.C. Bhandari, D.V.M., Ph.D., Principal Investigator

### **NTP Pathology Working Group**

*Evaluated slides, prepared pathology report (28 June 1990)*

J.C. Seely, D.V.M., Chair  
PATHCO, Inc.  
M.R. Elwell, D.V.M., Ph.D.  
National Toxicology Program  
J. Everitt, D.V.M.  
Chemical Industry Institute of Technology  
M.P. Jokinen, D.V.M.  
National Toxicology Program  
M.M. McDonald, D.V.M., Ph.D.  
National Toxicology Program

### **Biotechnical Services, Inc.**

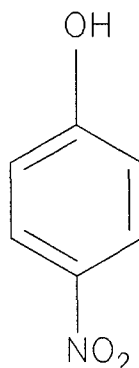
*Prepared Technical Report*

D.D. Lambright, Ph.D., Principal Investigator  
G.F. Corley, D.V.M.  
M.C. Hirrel, Ph.D.  
K.D. Mencer, B.A.

# CONTENTS

ABSTRACT .....	5
EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY .....	8
TECHNICAL REPORTS REVIEW SUBCOMMITTEE .....	9
SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS .....	10
INTRODUCTION .....	11
MATERIALS AND METHODS .....	15
RESULTS .....	21
DISCUSSION AND CONCLUSIONS .....	29
REFERENCES .....	31
APPENDIX A      Summary of Lesions in Male Mice in the 18-Month Dermal Study of <i>p</i> -Nitrophenol .....	35
APPENDIX B      Summary of Lesions in Female Mice in the 18-Month Dermal Study of <i>p</i> -Nitrophenol .....	85
APPENDIX C      Genetic Toxicology .....	135
APPENDIX D      Compound-Related Skin Lesions in Swiss-Webster Mice .....	145
APPENDIX E      Chemical Characterization and Dose Formulation Studies .....	149
APPENDIX F      Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration .....	157
APPENDIX G      Sentinel Animal Program .....	163

## ABSTRACT

***p*-NITROPHENOL**

CAS No. 100-02-7

Chemical Formula:  $C_6H_5NO_3$  Molecular Weight: 139.11**Synonyms:** 4-hydroxynitrobenzene, *p*-hydroxynitrobenzene, 4-nitrophenol, paranitrophenol, PNP, Niphen

*p*-Nitrophenol is used in the production of acetaminophen, methyl and ethyl parathion insecticides, fungicides, and dyestuffs. Toxicology and carcinogenesis studies of *p*-nitrophenol (greater than 97% pure) were conducted by dermal application to male and female Swiss-Webster mice for 18 months. Dermal application was selected as the route of chemical administration because of possible skin absorption from *p*-nitrophenol-treated leather footwear. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, and *Drosophila melanogaster*.

**18-MONTH STUDIES**

Groups of 60 Swiss-Webster mice of each sex received *p*-nitrophenol in acetone applied to the interscapular skin. Doses of 0, 40, 80, or 160 mg/kg *p*-nitrophenol were administered to mice 3 days per week for 78 weeks. At the end of the study, survival

rates of mice receiving 0, 40, 80, or 160 mg/kg *p*-nitrophenol were 29/60, 17/60, 26/60, and 24/60 for males and 35/60, 26/60, 33/60, and 27/60 for females.

Deaths after 60 weeks were caused by generalized amyloidosis and secondary kidney failure. The severity of amyloidosis was similar among dosed and control animals. At the end of the study, the final mean body weights of the dosed groups of each sex were similar to those of the controls. No biologically significant lesions were observed that were related to the dermal administration of *p*-nitrophenol.

**GENETIC TOXICOLOGY**

*p*-Nitrophenol was not mutagenic in *Salmonella typhimurium* (strains TA100, TA1535, TA1537, and TA98) with or without exogenous metabolic (S9) activation, or in germ cells of male *Drosophila melanogaster* administered *p*-nitrophenol in feed or by

injection. In Chinese hamster ovary cells, no induction of sister chromatid exchanges was observed with or without S9, but a significant increase in chromosomal aberrations occurred in trials conducted with S9.

## CONCLUSIONS

Under the conditions of these 18-month dermal studies there was *no evidence of carcinogenic activity*\* in male or female Swiss-Webster mice receiving 40, 80, or 160 mg/kg *p*-nitrophenol.

---

\*Explanation of Levels of Evidence of Carcinogenic Activity appears on page 8. A summary of Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 10.

Summary of the 18-Month Carcinogenicity and Genetic Toxicology Studies of *p*-Nitrophenol

Variable	Male Swiss-Webster Mice	Female Swiss-Webster Mice
Doses	0, 40, 80, or 160 mg/kg in acetone applied to the interscapular skin at a dose volume of 100 $\mu$ L	Same as male mice
Final body weights	Dosed groups similar to the controls	Dosed groups similar to the controls
18-Month survival rates	29/60, 17/60, 26/60, 24/60	35/60, 26/60, 33/60, 27/60
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Levels of evidence of carcinogenic activity	No evidence	No evidence
Genetic toxicology		
<i>Salmonella typhimurium</i> gene mutations:	Negative with or without S9 metabolic activation in strains TA100, TA1535, TA1537, and TA98	
Sister chromatid exchange		
Chinese hamster ovary cells <i>in vitro</i> :	Negative with or without S9 metabolic activation	
Chromosomal aberration		
Chinese hamster ovary cells <i>in vitro</i> :	Negative without S9 metabolic activation; positive with S9 metabolic activation	
Sex-linked recessive lethal mutations		
<i>Drosophila melanogaster</i> :	Negative when administered in feed or by injection	

## EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that because of major flaws cannot be evaluated (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983, and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity describes studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement is selected for a particular experiment, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS  
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on *p*-nitrophenol on July 9, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Subcommittee members have five major responsibilities:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Daniel S. Longnecker, M.D., Chair

Department of Pathology  
Dartmouth Medical School  
Hanover, NH

Jay I. Goodman, Ph.D.

Department of Pharmacology and Toxicology  
Michigan State University  
East Lansing, MI

Paul T. Bailey, Ph.D., Principal Reviewer

Toxicology Division  
Mobil Oil Corporation  
Princeton, NJ

David W. Hayden, D.V.M., Ph.D.

Department of Veterinary Pathobiology  
College of Veterinary Medicine  
University of Minnesota  
St. Paul, MN

Louis S. Beliczky, M.S., M.P.H., Principal Reviewer

Department of Industrial Hygiene  
United Rubber Workers International Union  
Akron, OH

Curtis D. Klaassen, Ph.D.

Department of Pharmacology and Toxicology  
University of Kansas Medical Center  
Kansas City, KS

Gary P. Carlson, Ph.D.

Department of Pharmacology and Toxicology  
Purdue University  
West Lafayette, IN

Barbara McKnight, Ph.D.

Department of Biostatistics  
University of Washington  
Seattle, WA

Harold Davis, D.V.M., Ph.D., Principal Reviewer

School of Aerospace Medicine  
Brooks Air Force Base, TX

Ellen K. Silbergeld, Ph.D.\*

University of Maryland Medical School  
Baltimore, MD

Robert H. Garman, D.V.M.

Consultants in Veterinary Pathology  
Murrysville, PA

Lauren Zeise, Ph.D.

Reproductive and Cancer Hazard Assessment Section  
California Environmental Protection Agency  
Berkeley, CA

\* Did not attend

## SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

Dr. C.C. Shackelford, NIEHS, introduced the toxicology and carcinogenesis studies of *p*-nitrophenol by discussing the uses of the chemical and the rationale for the study, describing the experimental design, reporting on survival and body weight effects, and commenting on the lack of compound-related neoplasms or nonneoplastic lesions in mice. The studies were terminated at 18 months because of reduced survival due to generalized amyloidosis and secondary kidney failure in both dosed and control animals. The proposed conclusions were *no evidence of carcinogenic activity* in male or female Swiss-Webster mice.

Dr. P.T. Bailey, a principal reviewer, agreed with the proposed conclusions. He questioned the sensitivity of Swiss-Webster mice to *p*-nitrophenol toxicity compared to that of other strains and whether parallels could be drawn between Swiss-Webster and other strains of mice used in classical carcinogenicity testing. Dr. Shackelford said there was no information on such studies in the literature. Dr. Bailey also asked whether clinical chemistry had been performed. Dr. Shackelford explained that at 65 weeks, when blood samples are normally taken for clinical chemistry, a large number of the animals were moribund and there were insufficient hematologic data to report.

Dr. H. Davis, the second principal reviewer, agreed with the proposed conclusions. He questioned the rationale for using Swiss-Webster mice. Dr. R. Irwin,

NIEHS, said that the U.S. Army specifically requested that the study be done in this strain.

Mr. L.S. Beliczky, the third principal reviewer, agreed with the proposed conclusions, although he considered the Swiss-Webster mouse a poor choice of animal, thus precluding any final judgment regarding either toxicity or carcinogenicity of *p*-nitrophenol. He said another study using a different strain of animal should be considered. Dr. S.L. Eustis, NIEHS, responded that the usual NTP rodent species, B6C3F<sub>1</sub> mouse and Fischer 344 rat, may not be the best strains to use for dermal carcinogenicity studies because they are relatively resistant to dermal carcinogenesis. He thought that other studies by another route in these strains would be more useful.

There was a lengthy discussion on the merits of exposure by the feed route versus dermal application and the point was made that when a chemical is given by one of these routes there is usually some degree of inadvertent exposure by the other route. Dr. R. Griesemer, NIEHS, commented that the design of the dermal study might be quite different depending on whether the concern was with skin as a target site or skin as a portal of entry.

Dr. Bailey moved that the Technical Report on *p*-nitrophenol be accepted with the conclusions as written for male and female Swiss-Webster mice, *no evidence of carcinogenic activity*. Dr. Davis seconded the motion, which was accepted unanimously with 10 votes.